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Basic semen examination — Specification and test methods

Analyse de base du sperme — Spécifications et méthodologie analytique

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Contents

Page

Foreword ..... **v**

Introduction ..... **vi**

1 Scope **1**

2 Normative References **1**

3 Terms and Definitions **1**

4 Staff Training and Competence **4**
4.1 General Aspects 4
4.2 Training 4
4.2.1 General 4
4.2.2 Training for quantitative assessments 4
4.2.3 Training for qualitative assessments 4
4.2.4 Training for pH assessment 4
4.3 Maintenance of Competence 5

5 Semen Characteristics, Sampling and Pre-Examination Handling **5**
5.1 General Characteristics 5
5.2 Physical and Chemical Characteristics 5
5.3 Sample Collection and Initial Handling 5
5.4 Subject Information and Data Collection 6
5.4.1 Information to be Provided to Subjects 6
5.4.2 Data Collection from the Subject 6
5.5 Initial Sample Handling 7
5.6 Sperm Toxicity Testing 7

6 Examinations **7**
6.1 Required Equipment 7
6.2 In-house Prepared Reagents 8
6.3 Assessments 8
6.3.1 Initiation of Assessments 8
6.3.2 Macroscopic Assessment 9
6.3.3 Direct Microscopy of the Wet Preparation 9
6.3.4 Sperm Motility Assessment 9
6.3.5 Sperm Concentration Assessment 10
6.3.6 Assessment of Absence of Spermatozoa 10
6.3.7 Sperm Vitality Assessment 11
6.3.8 Sperm Morphology Evaluation 11

7 Post-Examination Handling and Test Report **11**
7.1 General 11
7.2 Results Calculations and Presentation 11
7.2.1 Total Amount in the Ejaculate 11
7.2.2 Other Calculations 11
7.3 Presentation of Results 12
7.3.1 General 12
7.3.2 Contents of the Semen Examination Report 12
7.4 Practical Aspects of Quality Assurance 13
7.4.1 Internal Quality Control 13
7.4.2 Intralaboratory Comparisons 13
7.4.3 Interlaboratory Comparisons 14

Annex A (informative) **The statistical basis for determination of absence of spermatozoa** **15**

Annex B (informative) **High power field** **16**

Annex C (informative) **Motility assessment training** **17**

Annex D (informative) **Diluent for sperm concentration assessment** **20**

Annex E (informative) **Estimation of suitable dilution for the assessment of sperm concentration** **21**
Annex F (informative) **Comparison of concordance between two replicate assessments that report percentages** **22**
Annex G (informative) **Comparison of concordance between two replicate counts of sperm concentration** **24**
Annex H (informative) **Sperm vitality assessment** **27**
Annex I (informative) **Sperm morphology assessment** **28**
Bibliography **31**

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document was developed in response to global demand for standards for reliable examination of human semen. The five editions of a laboratory manual for human semen analysis published by the WHO between 1980 and 2010 have provided general recommendations for suitable laboratory procedures, but even the latest edition (World Health Organization 2010 [16]) does not constitute a Technical Standard adequate for use under ISO 15189.

A Technical Standard based on best available evidence and global consensus regarding laboratory procedures most likely to give reliable results will facilitate any laboratory seeking accreditation for human semen examination. Subjects, and biomedical science in general, would benefit from fewer random factors affecting the accuracy of results. Clinically this would support improved diagnoses as well as provide more objective grounds for choosing between possible management strategies or alternative treatment modalities. Furthermore, to support the evaluation and validation of new methods to improve the diagnosis and treatment of infertility, these standardized techniques can serve as reference methods.

The pre-examination preparation of human semen is important not only in manual basic semen examination, but also for Computer-Aided Sperm Analysis (CASA). Standardized handling and preparation of semen samples is essential to the quality of the data obtained.

Basic semen examination — Specification and test methods

1 Scope

This document specifies the minimum requirements for equipment and critical aspects of the test methods for best practice in laboratories performing basic examination of human semen collected by ejaculation.

This document is applicable to the entire process of basic manual semen examination and also to sample preparation for Computer-Aided Sperm Analysis (CASA).

This document does not apply to the post-vasectomy assessments.

NOTE Given the medico-legal ramifications surrounding the evaluation of post-vasectomy ejaculates, the methodology in this document is in all likelihood inadequate to establish an ejaculate as being completely "clear" (i.e. no spermatozoa in the ejaculate).

2 Normative References

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 15189, *Medical laboratories — Requirements for quality and competence*

ISO/TS 20914, *Medical laboratories — Practical guidance for the estimation of measurement uncertainty*

3 Terms and Definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
air displacement pipette
common laboratory pipette with disposable tips where the volume aspirated is controlled by the displacement of an equivalent volume of air inside an enclosed chamber inside the pipette handle

Note 1 to entry: An air displacement pipette can only give accurate volumes for liquids with viscosity close to that of water.

3.2
azoospermia
complete absence of spermatozoa in the *ejaculate* (3.4)

Note 1 to entry: The term azoospermia is not a clinical diagnosis but a description of a laboratory finding. Complete lack of spermatozoa is difficult to determine in absolute terms. Since only parts of an *ejaculate* (3.4) can be examined, the modern definition is based on probability calculations derived from data obtained from investigations of random aliquots from an *ejaculate* (3.4) (See Annex A).

3.3
CASA
computer-aided sperm analysis
automated examination of *ejaculates* (3.4) with equipment using imaging technology

Note 1 to entry: Examination based on image analysis of video sequences to obtain information on *sperm concentration* (3.18) and motility, more seldom sperm morphology.

Note 2 to entry: There are CASA systems commercially available, but no common standard for validation, evaluation, reliability in analyses or contents of reports. The scope of this document is not to provide a standard for CASA, although the pre-examination aspects can be useful also to developers, manufacturers, and users of CASA equipment.

3.4
ejaculate
semen sample, which is a mixture of spermatozoa and secretions, mainly from the seminal vesicles, the prostate and the epididymides

Note 1 to entry: The ejaculate can be obtained by various methods including masturbation, intercourse, vibratory stimulation or electro-ejaculation.

3.5
ejaculate viscosity
property of an *ejaculate* (3.4) describing its resistance to flow like water after *liquefaction* (3.10)

Note 1 to entry: Incompletely liquefied semen is not a homogenous liquid due to the contents of gelatinous structures in the ejaculate fluid.

3.6
high power field
area of a slide which is visible in the microscope under high power magnification (×400)

Note 1 to entry: This is not a standard field area as the size varies according to the type of oculars used (e.g. standard or wide field) (see Annex B).

3.7
immotile
total lack of active tail movements

3.8
interlaboratory comparison
organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions

[SOURCE: ISO/IEC 17043:2010, 3.4]

3.9
ideal spermatozoon
spermatozoon with the morphology typical of spermatozoa able to penetrate into and migrate within cervical mucus and reach the site of fertilization

[SOURCE: Menkveld, et al., 1991,[9] Menkveld and Kruger, 1995[10]]

3.10
liquefaction
process of change in the consistency of the *ejaculate* (3.4) from gel-like or coagulum-like into a liquid phase

Note 1 to entry: Liquefaction occurs due to degradation of the gel-like or coagulum-like property, by enzymatic action on macromolecules.

3.11
non-progressive sperm motility
active tail movements leading to a sperm propagation of less than approximately 5 µm/s

Note 1 to entry: A normal head length is approximately 5 µm.

3.12
positive displacement pipette
common laboratory pipette working by piston-driven displacement within a capillary, not the displacement of air within an enclosed chamber

Note 1 to entry: The piston in the pipette tip is in direct contact with the liquid specimen.

Note 2 to entry: Use to avoid major volume errors with viscous liquids like semen.

3.13
progressive sperm motility
forward motility of a spermatozoon of at least 5 µm/s

Note 1 to entry: See also *slow progressive sperm motility* (3.16) and *rapid progressive sperm motility* (3.14).

Note 2 to entry: Spermatozoa moving in circular paths are considered progressive based on space gain.

3.14
rapid progressive sperm motility
forward motility of a spermatozoon of at least 25 µm/s

3.15
sexual abstinence
time between the collection of *ejaculate* (3.4) for analysis and the most recent previous ejaculation

Note 1 to entry: Expressed in days or hours as appropriate for the intended use.

3.16
slow progressive sperm motility
forward motility of a spermatozoon of at least 5 µm/s but less than 25 µm/s

3.17
specimen collection container
receptacle used to collect primary samples

Note 1 to entry: Specimen collection container shall be not toxic to spermatozoa.

Note 2 to entry: If an *ejaculate* (3.4) can only be collected at sexual intercourse, a non-toxic, Silastic™ condom can be used. The *ejaculate* (3.4) shall be transferred to an ejaculate sample container upon receipt by the laboratory; this shall be noted in the report form.

3.18
sperm concentration
number of spermatozoa per unit volume

Note 1 to entry: Sperm concentration is expressed in millions or thousands/millilitre.

Note 2 to entry: It shall not be confused with sperm density (mass/volume).

3.19
sperm vitality
percentage of vital spermatozoa, independent of their ability to move

3.20
total sperm number
calculated total number of spermatozoa in the *ejaculate* (3.4)

Note 1 to entry: Total sperm number is the *sperm concentration* (3.18) multiplied by the *ejaculate* (3.4) volume.

Note 2 to entry: Total sperm number is not the same as *sperm concentration* (3.18).

3.21
Tygerberg strict criteria
sperm morphology criteria based on the morphology of spermatozoa able to penetrate into and migrate within cervical mucus

3.22
Teratozoospermia Index
TZI
average number of defective regions (head, neck/midpiece, tail, and/or cytoplasmic droplet) in abnormal spermatozoa

Note 1 to entry: This index is, by definition, never outside the interval of [1.00;4.00].

4 Staff Training and Competence

4.1 General Aspects

General requirements for staff training and competence are covered in ISO 15189. How these requirements are applied to human semen analysis is covered here.

4.2 Training

4.2.1 General

Semen examination involves many analytical steps that require operator training to minimize subjectivity in order to provide accurate reliable results[7][12][1].

4.2.2 Training for quantitative assessments

All assessors performing assessments of sperm motility, sperm concentration, sperm vitality and/or sperm morphology shall receive training using either commercial, in-house or EQA-derived validated reference materials to ensure that their results conform to the laboratory's pre-determined measurement error limits. Without such training staff cannot be expected to be able to provide accurate or reliable results for these assessments, and participation in EQA schemes is pointless.

NOTE Effective goal-oriented reiterative training procedures for these assessments have been published[12][14]; a ± 10 % range of measurement error is expected between novices upon completion of their training and the laboratory's experienced staff (see also Annex C).

4.2.3 Training for qualitative assessments

Competency training for qualitative assessments, such as viscosity and round cells, shall achieve agreement between trainee and expert in at least 90 % of cases.

4.2.4 Training for pH assessment

The ability of assessors to read test strips against the comparator scale shall be verified.

4.3 Maintenance of Competence

Ongoing verification of competence shall be demonstrated by all personnel performing these assessments at regular intervals as defined in the laboratory's quality framework.

NOTE According to 4.2, the same ± 10 % range of measurement error is expected for ongoing verification of competence by all trained staff performing these assessments.

5 Semen Characteristics, Sampling and Pre-Examination Handling

5.1 General Characteristics

Examination of the ejaculate is in some important aspects different from investigations of other human bodily fluids. The subject is expected to accomplish the collection of the ejaculate. Results are dependent on ejaculation frequency before collection, as well as on the time and temperature before initiation of investigations. In case of infertility diagnosis, clear reference limits are missing due to the fact that the desired outcome is dependent on the particular clinical situation of each couple trying to achieve a pregnancy.

5.2 Physical and Chemical Characteristics

There is no internal homeostatic control in an ejaculate collected in a device for laboratory investigations. Initially the entire ejaculate is incorporated into a gel-like coagulum that is gradually degraded (liquefaction) into a still viscous but more water-like liquid. During this process carbon dioxide evaporates causing a change in pH. Enzymatic degradation of gel components causes a significant increase in osmotic properties of the liquid surrounding the spermatozoa, which in turn affects sperm performance.

5.3 Sample Collection and Initial Handling

Sample collection shall, except for some men with, for example, disabled limbs, spinal cord injury or paraplegia, always be done by the subject. If necessary, the subject's partner can help with sample collection. For subjects with ethical or religious objections to masturbation a non-spermotoxic (Silastic™[1]) condom can be used to collect an ejaculate during intercourse. However, this collection method will result in some loss of the overall sample as it is recovered from the condom. Collection of ejaculates by coitus interruptus ("withdrawal") is not recommended as the first, sperm-rich, fraction of the ejaculate is often lost. Use of lubricants can be necessary by some subjects; such products shall be validated as non-toxic to spermatozoa[13].

After ejaculation, the sample shall be kept as close as possible to 37 °C and never higher; cooling or warming can cause artefacts and sperm dysfunction. Due to all the changes occurring after ejaculation, investigations shall start as soon as possible after liquefaction, that typically is completed within 30 min after ejaculation. Incomplete liquefaction at 60 min after ejaculation indicates an abnormality. Initiation of assessments after completion of liquefaction is best achieved if the ejaculate is collected near the laboratory. Since the duration and level of sexual arousal experienced by the subject will affect the ejaculation, sample collection could be best performed in a place chosen by the subject in case of major difficulty. When an ejaculate is collected outside the lab environment it shall be delivered to the laboratory, preferably within 30 min, but at least within 60 min (circumstance for ejaculate collection and transport shall be noted in the report). Nonetheless, considerations of temperature and time to investigation remain important for the quality and robustness of the examination.

1) Silastic™ is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

5.4 Subject Information and Data Collection

5.4.1 Information to be Provided to Subjects

The following information shall be provided to the subject in writing in a language understandable by the subject and shall include the following:

a) General information:

- Contact information for the laboratory;
- The reason for the investigation if made available from the requester;
- An outline of what will be investigated;
- How results of the laboratory investigations will be communicated to the subject.

b) Ejaculate collection, handling and transportation:

- How to collect the ejaculate;
- Effect of delay between sample collection and initiation of assessments;
- Importance of avoiding cooling down or warming up of the ejaculate;
- Importance of reporting correct sexual abstinence time;
- Importance of reporting any incompleteness of sample collection.

5.4.2 Data Collection from the Subject

a) Required information

Each subject shall be asked to provide the following information to be recorded by the laboratory:

- Reliable personal ID;
- Duration of sexual abstinence;
- Time of sample collection;
- Transport of ejaculates should be avoided but if not collected at the premises of the laboratory: confirmation that during transport to the laboratory the specimen was protected from extremes of temperature;
- Completeness of sample collection; in case of incomplete collection, with information of which parts in the sequence of ejaculation that have been missed in collection.

b) **Additional information**

The information is of importance to the clinical interpretation and can be practical to obtain when the subject visits the laboratory but is not part of the laboratory work.

- Medical history, which can include:
  - Any episode of severe inflammatory process the last three months;
  - Any previous surgery (inguinal hernia, varicocele, cryptorchidism or other problems related to the urogenital sphere) or treatment with chemotherapy, cytostatics or radiation of the urogenital organs;

  - Any use of pharmaceutical drugs except short term use of non-prescription drugs (e.g. pain killers, and anti-allergy drugs).

- Any use of recreational drugs, anabolic steroids or other performance enhancing dietary additions (like protein powders).

5.5 Initial Sample Handling

- Every ejaculate should be considered potentially infectious and handled accordingly (see ISO 15190:2020, Annex B).

- Information provided by the subject shall be recorded.

- Specimen collection container shall be clean, non-toxic and for single use.

- Specimen collection container should preferably be weighed before sample collection and its weight recorded with two decimal places.

- Ejaculate volume should preferably be determined by weight. In this case the specimen collection container is weighed before and after specimen collection and the weight difference used as the volume, assuming 1,0 g of ejaculate equals 1.0 ml of ejaculate[3]. If a calibrated serological pipette is used, some semen will always be lost in the specimen collection container and inside the pipette after making the measurement. The lab should be aware of the differences of the two methods. The ejaculate volume shall be reported in ml to one decimal place.

- All documents and the specimen collection container shall be labelled with at least two unique identifiers.

- As soon as possible after collection the specimen collection container shall be kept at a temperature between 35 °C and 37 °C to facilitate liquefaction and prepare for motility assessment at standardized temperature, preferably on a moving tray to enhance mixing during liquefaction (frequent manual agitation is required when moving tray is not available).

Spermatozoa are affected by the earth gravity and sediment to the bottom of any container (“geotaxis”) even if they are motile. Consequently, when sampling an ejaculate, it shall be well-mixed to evenly distribute the spermatozoa and other elements of the ejaculate. Even sitting for a short period of time will result in an uneven distribution of the cellular elements of the ejaculate. It is therefore important to gently mix the ejaculate thoroughly before any aliquot is taken for examination, noting that a vortex mixer shall not be used.

5.6 Sperm Toxicity Testing

To ensure that materials in contact with ejaculates (specimen collection container, pipette tips) are not toxic to spermatozoa, a basic toxicity test shall be performed on every new batch of material. The principle is based on comparison of motility of spermatozoa exposed to present material and the new material[6]. The time of exposure shall be at least twice the expected time of exposure of sperm to the material – seconds for pipette tips and 30 min to 60 min for sample collection containers.

6 Examinations

6.1 Required Equipment

Sperm motility is largely influenced by the ambient temperature, especially regarding velocity. The use of temperature-controlled equipment reduces the influence of variable room temperature.

The following equipment is required:

- Laboratory balance, range 0,00 g to 50,00 g (reading to two decimal places);